

Abundance-weighted phylogenetic diversity measures distinguish microbial community states and are robust to sampling depth

Connor O. McCoy and Frederick A. Matsen IV

Fred Hutchinson Cancer Research Center, Seattle, WA, United States

ABSTRACT

In microbial ecology studies, the most commonly used ways of investigating alpha (within-sample) diversity are either to apply non-phylogenetic measures such as Simpson's index to Operational Taxonomic Unit (OTU) groupings, or to use classical phylogenetic diversity (PD), which is not abundance-weighted. Although alpha diversity measures that use abundance information in a phylogenetic framework do exist, they are not widely used within the microbial ecology community. The performance of abundance-weighted phylogenetic diversity measures compared to classical discrete measures has not been explored, and the behavior of these measures under rarefaction (sub-sampling) is not yet clear. In this paper we compare the ability of various alpha diversity measures to distinguish between different community states in the human microbiome for three different datasets. We also present and compare a novel one-parameter family of alpha diversity measures, $BWPD_{\theta}$, that interpolates between classical phylogenetic diversity (PD) and an abundance-weighted extension of PD. Additionally, we examine the sensitivity of these phylogenetic diversity measures to sampling, via computational experiments and by deriving a closed form solution for the expectation of phylogenetic quadratic entropy under re-sampling. On the three datasets, a phylogenetic measure always performed best, and two abundance-weighted phylogenetic diversity measures were the only measures ranking in the top four across all datasets. OTU-based measures, on the other hand, are less effective in distinguishing community types. In addition, abundance-weighted phylogenetic diversity measures are less sensitive to differing sampling intensity than their unweighted counterparts. Based on these results we encourage the use of abundance-weighted phylogenetic diversity measures, especially for cases such as microbial ecology where species delimitation is difficult.

Submitted 1 May 2013
Accepted 20 August 2013
Published 12 September 2013

Corresponding author
Frederick A. Matsen IV,
matsen@fhcrc.org

Academic editor
Ludmila Chistoserdova

Additional Information and
Declarations can be found on
page 14

DOI 10.7717/peerj.157

© Copyright
2013 McCoy and Matsen

Distributed under
Creative Commons CC-BY 3.0

OPEN ACCESS

Subjects Biodiversity, Bioinformatics, Ecology, Microbiology, Statistics

Keywords Alpha diversity, Diversity indices, Phylogenetic diversity, Microbial ecology, Human microbiome

INTRODUCTION

It is now well accepted that incorporating phylogenetic information into alpha (single-sample) and beta (between-sample) diversity measures can be useful in a variety of ecological contexts. Phylogenetic equivalents of all of major alpha diversity measures have

Table 1 Overview of phylogenetic diversity measures used in the text.

phylogenetic diversity (<i>Faith, 1992</i>)	<i>phylogenetic generalization of species count</i>
phylogenetic quadratic entropy (<i>Rao, 1982; Warwick & Clarke, 1995</i>)	<i>phylogenetic generalization of the Simpson index</i>
phylogenetic entropy (<i>Allen, Kon & Bar-Yam, 2009</i>)	<i>phylogenetic generalization of the Shannon index</i>
${}^qD(T)$ (<i>Chao, Chiu & Jost, 2010</i>)	<i>phylogenetic generalization of Hill numbers</i>
BWPD ₁ (<i>Barker, 2002; Vellend et al., 2011</i>)	<i>abundance-weighted version of phylogenetic diversity</i>
BWPD _{θ} (this paper)	<i>one-parameter family interpolating between PD and BWPD₁</i>

been developed (Table 1). Starting with Faith’s original definition of phylogenetic diversity (*Faith, 1992*), which generalizes species count, there are now phylogenetic generalizations of the Simpson index to Rao’s quadratic entropy (*Rao, 1982; Warwick & Clarke, 1995*), the Shannon index to phylogenetic entropy (*Allen, Kon & Bar-Yam, 2009*), and the Hill numbers to ${}^qD(T)$ (*Chao, Chiu & Jost, 2010*). Phylogenetic diversity itself has been extended to incorporate taxon counts (*Barker, 2002*) and proportional abundance (*Vellend et al., 2011*). There have also been abundance-weighted measures that explicitly measure phylogenetic community structure (*Fine & Kembel, 2011*), or an “effective number of species” (*Chao, Chiu & Jost, 2010*). Many diversity measures can be tidily expressed in the framework of *Leinster & Cobbold (2012)*, although the expression of phylogenetic diversity measures for non-ultrametric trees is complex.

In this paper we use three example human microbiome datasets to demonstrate the utility of abundance-weighted phylogenetic diversity measures. We also introduce a one-parameter family interpolating between classical PD and an abundance-weighted generalization. We call the parameter θ and denote the one-parameter family BWPD _{θ} ; BWPD₀ is classical PD, whereas BWPD₁ is balance-weighted phylogenetic diversity, effectively PD_{aw} of *Vellend et al. (2011)*. Intermediate values of θ allow a partially-abundance-weighted compromise. Such a compromise has recently been shown to be useful for measuring beta diversity, with the introduction of a one-parameter family of “generalized UniFrac” measures (*Chen et al., 2012*). We use the name Balance Weighted Phylogenetic Diversity as described below because there are a variety of abundance weighted phylogenetic diversity measures. We compare the behavior of PD measures, including BWPD _{θ} , under various levels of sampling using theory and example datasets.

MATERIALS AND METHODS

Datasets

We apply the methods described below to three previously described 16S rRNA surveys of the human microbiome. The first two datasets are composed of samples from “normal” and dysbiotic microbial communities, where previous studies have associated changes

in diversity with changes in health. The third dataset investigates the changes of the skin microbiome through time.

Bacterial vaginosis

First, we reanalyze a pyrosequencing dataset describing bacterial communities from women being monitored in a sexually transmitted disease clinic for bacterial vaginosis (BV). BV has previously been shown to be associated with increased community diversity ([Fredricks, Fiedler & Marrazzo, 2005](#)). For this study, swabs were taken from 242 women from the Public Health, Seattle and King County Sexually Transmitted Diseases Clinic between September 2006 and June 2010 of which 220 samples resulted in enough material to analyze ([Srinivasan et al., 2012](#)).

Selection of reference sequences and sequence preprocessing were performed using the methods described in [Srinivasan et al. \(2012\)](#). 452,358 reads passed quality filtering, with a median of 1,779 reads per sample (range: 523–2,366).

Oral periodontitis

We also utilize sequence data from a study of subgingival communities in 29 subjects with periodontitis, along with an equal number of healthy controls ([Griffen et al., 2011a](#)). The publication analyzing this dataset showed increased community diversity in samples from dysbiotic patients compared to healthy controls. Raw sequences were filtered, retaining only those reads with: a mean quality score of at least 25, no ambiguous bases, at least 150 base pairs in length, and an exact match to the sequencing primer and barcode. A total of 759,423 reads passed quality filtering, with a median of 8,320 reads per sample (range: 4,096–14,319).

As the phylogenetic placement method used below to calculate our measures requires a reference tree and alignment, we created a tree with FastTree 2.1.4 ([Price, Dehal & Arkin, 2010](#)) using the alignment and accompanying taxonomic annotation from the curated CORE database of oral microbiota ([Griffen et al., 2011b](#)).

Skin microbiome through time

Our third dataset is a study of skin microbial diversity through adolescence ([Oh et al., 2012](#)). Aligned sequences were obtained courtesy of the authors, although sequence data is available under the accession numbers [GQ000001] to [GQ116391] and can be accessed through BioProject ID 46333. A total of 90,142 Sanger sequences were available, with a median of 693 sequences per sample (range: 317–2884).

Balance-weighted phylogenetic diversity

In this section we introduce $BWPD_{\theta}$, our one-parameter family interpolating between classical PD and fully balance-weighted phylogenetic diversity. We will primarily consider so-called *unrooted* ([Pardi & Goldman, 2007](#)) phylogenetic diversity, which does not necessarily include the root. The case of *rooted* phylogenetic diversity can be calculated in a similar though simpler way as described below. Although we will primarily be working in an unrooted sense, it will be useful to use terminology that corresponds to the rooted case. For this reason, if the tree is not already rooted, assume an arbitrary root has been

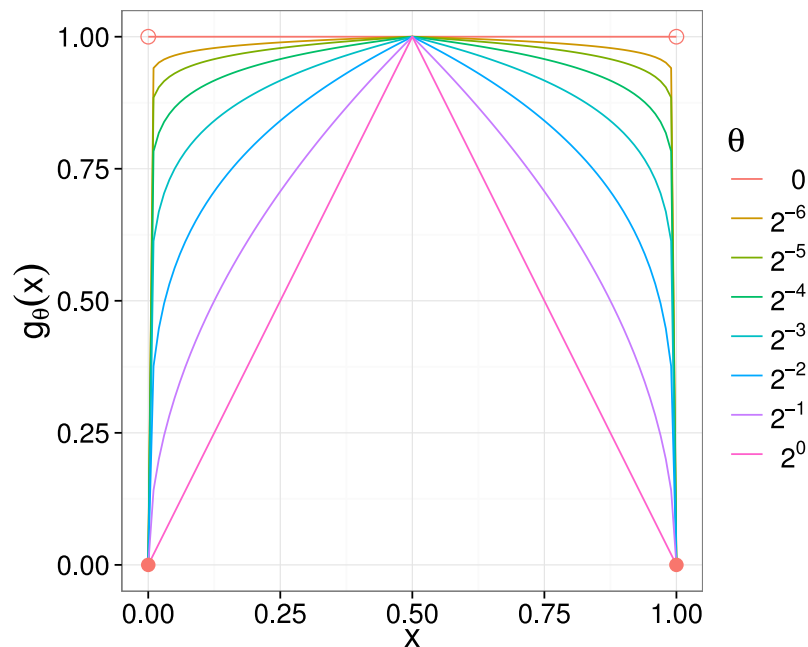


Figure 1 g_θ curves for various θ parameters. As θ goes to zero, the g_θ converge pointwise to g , which is 1 on the interior of the unit interval and 0 on the boundaries.

chosen; let the *proximal* side of a given edge be the side that contains the root and *distal* be the other.

We will describe BWPD_θ in terms of a phylogenetic tree T with leaves L , and a *contingency table* describing the number of observations of the organisms at the leaves in various samples. The contingency table has rows labeled with the leaves of T , and columns labeled by samples. In microbial ecology this is frequently known as an *OTU table*. The entry corresponding to a given leaf and a given sample is the number of times that leaf was observed in that sample.

The classical (unrooted) phylogenetic diversity of a given sample in this context is the total branch length of the tree subtended by the leaves in that sample.

The path to generalizing PD is to note that this can be expressed as a sum of branch lengths multiplied by a step function. Let $f(x)$ be the function that is one for $x > 0$ and zero otherwise. Let $g(x) = \min(f(x), f(1-x))$ and $D_s(i)$ be the fraction of reads in sample s that are in leaves on the distal side of edge i . Phylogenetic diversity can be then expressed as

$$\text{PD}_u(s) = \sum_i \ell_i g(D_s(i)). \quad (1)$$

That is, the sum of edge lengths in T which have reads from s on both the distal and proximal side.

Note that the step function g is the limit of a one-parameter family of functions (Fig. 1). Indeed, defining

$$g_\theta(x) = [2 \min(x, 1-x)]^\theta, \quad (2)$$

g is the pointwise limit of the g_θ on the closed unit interval as θ goes to zero. Thus our one-parameter generalization is

$$\text{BWPD}_\theta(s) = \sum_i \ell_i g_\theta(D_s(i)). \quad (3)$$

Note that when $\theta = 0$ this is PD and when $\theta = 1$ this is an abundance-weighted version of PD equivalent to executing the Δ nPD recipe of [Barker \(2002\)](#) up to a multiplicative factor.

The rooted equivalent of (3) is

$$\text{RBWPD}_\theta(s) = \sum_i \ell_i (D_s(i))^\theta, \quad (4)$$

which interpolates between rooted PD and an abundance-weighted version. [Vellend et al. \(2011\)](#) describe a measure, PD_{aw} , which is equal to RBWPD_1 multiplied by the total number of branches in T .

We call BWPD_1 balance-weighted phylogenetic diversity because it weights edges according to the balance of read fractions on either side of an edge—edges with even amount of mass on either side are up-weighted, while edges with an uneven balance of mass are down-weighted. Indeed, if $|x - (1 - x)|$ is thought of as the imbalance of read fraction on either side of an edge, then $1 - |x - (1 - x)|$ is a measure of balance; note that on the unit interval, $2 \min(x, 1 - x) = 1 - |x - (1 - x)|$. Because a small x or an x close to 1 gives a small coefficient in the summation, small collections of reads or small perturbations of the read distribution will not change the value of BWPD_1 appreciably.

Calculation of PD measures in example applications

Reads from the vaginal and oral studies were placed on a tree created from a curated set of taxonomically annotated reference sequences. As phylogenetic entropy and ${}^q\text{D}(T)$ operate on a rooted phylogeny, reference trees were assigned a root taxonomically ([Matsen & Gallagher, 2012](#)) meaning that a root was found that best separated high-level taxonomic groupings. `pplacer` was run in posterior probability mode (using the `-p` and `--informative-prior` flags), which defines an informative prior for pendant branch lengths with a mean derived from the average distances from the edge in question to the leaves of the tree. The resulting set of placements were classified at the family rank using a hybrid classifier implemented in the `guppy` tool from the `pplacer` suite. The hybrid classifier assigns taxonomic annotations to sequences using the combination of a naïve Bayes classifier ([Wang et al., 2007](#)) with a phylogenetic classifier (N Hoffman, A Gallagher and F Matsen, unpublished data). Any reads that could not be confidently classified to the family rank were not used in measures based on classification.

Full-length 16S sequences were available for the skin data, and so a more traditional tree-building approach was used. Representative OTUs were chosen for each site by clustering at 97% identity using `USEARCH 5.1` ([Edgar, 2010](#)), with trees built on OTU centroids using `FastTree` ([Price, Dehal & Arkin, 2010](#)). To conform with methods used in [Oh et al. \(2012\)](#), the naïve Bayes classifier ([Wang et al., 2007](#)) was used to infer genus-level classifications to taxonomically root the tree; in our case we used the RDP classifier v2.5.

The contingency (OTU) tables generated by clustering were made available to our tools via the BIOM (McDonald *et al.*, 2012) format.

PD_u (unrooted PD), phylogenetic quadratic entropy (Rao, 1982), phylogenetic entropy (Allen, Kon & Bar-Yam, 2009), and ⁴D(T) (Chao, Chiu & Jost, 2010) were implemented for phylogenetic placements in the freely-available **pplacer** suite of tools (Matsen, Kodner & Armbrust, 2010) (<http://matsen.fhcrc.org/pplacer>) in the subcommand **guppy fpd**. Prior to diversity estimation, either phylogenetic placements were rarefied to the read count of the specimen in the dataset with the fewest sequences using **guppy rarefy**, or the corresponding rarefaction on full-length sequences was performed with the QIIME (Caporaso *et al.*, 2010) rarefaction tool **single_rarefaction.py**. The mean value of each statistic over 100 such rarefactions was used for analysis.

Discrete measures of alpha diversity and richness were calculated on contingency tables obtained from clustering and taxonomic classification. Sequences were clustered into Operational Taxonomic Units (OTUs) at a 97% identity threshold using USEARCH 5.1 (Edgar, 2010). Similar results were observed when clustering at 95% identity (results not shown). OTU counts and family-level taxon counts were then rarefied as above in R 3.0.1 (R Development Core Team, 2013) using the **vegan** package (Oksanen *et al.*, 2012). We obtained values for the *Simpson (1949)* and *Shannon (1948)* diversity indices, as well as the Chao1 (Chao, 1984) and ACE (Chao & Lee, 1992) measures of species richness using **vegan** functions **diversity** and **estimateR**.

Comparative analysis of alpha diversity measures

To investigate the relation between various measures of alpha diversity, we calculated Pearson's *r* between all pairs of measures using the function **rcorr** from the R package **Hmisc** (Harrell, 2012). We then performed hierarchical clustering with the R function **hclust**, using $d = 1 - r$ as the distance between two measures.

Association of each measure with clinical criteria for the first two datasets was evaluated by examining the accuracy of a logistic regression using the measure as the sole predictor of whether the sample came from a "normal" or dysbiotic subject. In the vaginal dataset, we assessed each measure's ability to predict whether a sample was from a subject positive for BV by Amsel's criteria, a clinical diagnostic method (Amsel *et al.*, 1983). In the oral dataset, we assessed each measure's ability to predict whether a sample was from a healthy control, or a subject with periodontitis. Accuracy in predicting sample community state was assessed by leave-one-out cross-validation using the R package **boot** (Davison & Hinkley, 1997; Canty & Ripley, 2012).

For the vaginal dataset, we also calculated R^2 values using each measure individually as a predictor for sample Nugent score in a linear regression. The Nugent score provides a diagnostic score for BV which ranges from 0 (BV-negative) to 10 (BV-positive) based on presence and absence of bacterial morphotypes as viewed under a microscope (Nugent, Krohn & Hillier, 1991).

We calculated *p*-values to compare within- and between-stratification variability using R's built-in **t.test** function for the vaginal data, which had a binary stratification, and the

Table 2 Correlation and predictive performance of the various alpha diversity measures applied to the vaginal dataset. Rows are ordered by increasing mean rank across performance measurements. Nugent R^2 : R^2 value using the measure as a predictor, and the Nugent score as response in a linear model. Amsel accuracy: proportion of specimens with correct BV classification under a leave-one-out cross-validation. Amsel p -value: p -value from a two-sample t -test on values stratified by BV classification. “OTU” designates the measure applied to 97% clustering groups, and “Family” designates taxonomic classification at the family level. Measures described in main text.

Measure	Amsel accuracy	Nugent R^2	Amsel p -value	Mean rank
PD _u	0.834	0.737	1.84E−35	1.3
BWPD _{0.25}	0.836	0.735	1.98E−35	2.0
Simpson (Family)	0.817	0.735	4.11E−33	4.0
BWPD _{0.5}	0.827	0.700	3.33E−33	4.3
Shannon (Family)	0.813	0.724	2.28E−32	5.3
Phylo. entropy	0.831	0.679	1.81E−31	5.7
Chao1 (Family)	0.813	0.704	6.27E−31	7.0
^{0.5} D(T)	0.818	0.658	7.47E−29	8.0
^{0.25} D(T)	0.809	0.682	2.25E−30	8.3
Phylo. quad. entropy	0.813	0.648	7.89E−30	9.0
BWPD ₁	0.795	0.611	5.38E−28	11.0
Chao1 (OTU)	0.766	0.488	1.64E−23	12.7
ACE (Family)	0.766	0.491	2.82E−11	13.0
ACE (OTU)	0.764	0.469	6.82E−22	13.7
Shannon (OTU)	0.758	0.380	5.27E−16	14.7
Simpson (OTU)	0.697	0.191	1.42E−07	16.0

aoV function for the oral and skin datasets. The vaginal dataset was stratified by Amsel’s criterion, the oral dataset by condition and sampling site, and the skin microbiome dataset by Tanner scale of physical development (*Oh et al., 2012*). Note that we are not presenting these uncorrected p -values as evidence that there is an interesting relationship between the microbiome and a given stratification, but rather are using p -values as a way of measuring within-stratum heterogeneity compared to between-stratum heterogeneity for the various measures.

RESULTS

Application to the human microbiome

Vaginal microbiome

Like *Srinivasan et al. (2012)* and many others in the field, we observe greater diversity in BV positive specimens using a variety of diversity and richness measures (*Fig. S1*). In particular, this is true for BWPD _{θ} for a variety of values of θ (*Fig. S2*).

In the vaginal data, phylogenetic measures of alpha diversity have better cross-validation accuracy for the Amsel classification and better correlation with the Nugent score than discrete OTU-based measures (*Table 2*). All measures were somewhat accurate in identifying community state, with even the worst performers classifying almost 70% of

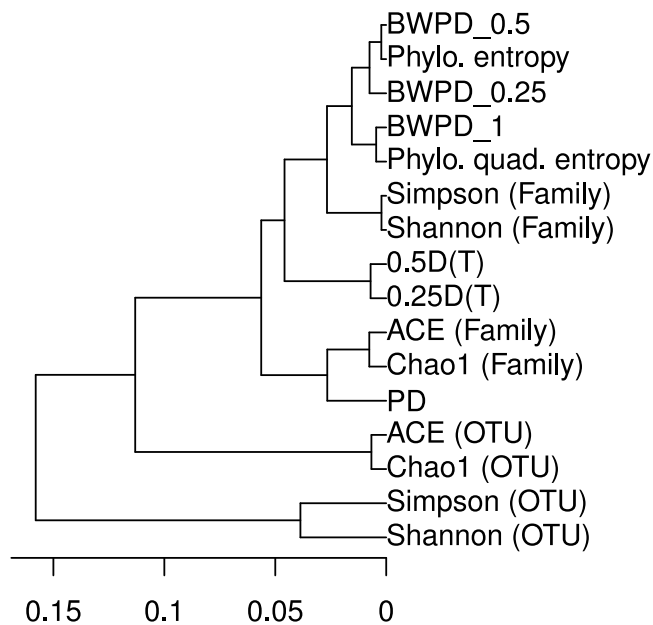


Figure 2 Dendrogram relating alpha diversity measures applied to the vaginal dataset.

samples correctly. $BWPD_{0.25}$, $BWPD_{0.5}$, PD_u , and phylogenetic entropy perform well predicting BV status. Correlation with Nugent score varies from 0.19 using Simpson (OTU) to 0.74 using PD_u . OTU-based measures rank in the bottom half of the measures tested, and below all phylogenetic measures.

In the hierarchical clustering of alpha measures on the vaginal dataset, phylogenetic methods are separated from OTU-based methods (Fig. 2). $BWPD_\theta$ is similar to different extant phylogenetic alpha diversity measures for different θ . The Simpson and Shannon diversity measures cluster together, as do the ACE and Chao1 richness measures.

Figure 3 shows values of $BWPD_\theta$ calculated before (x -axis) and after (y -axis) a single rarefaction to 523 sequences per sample. Samples for which the $BWPD_\theta$ value changes little lie close to the blue line, which shows the case of no difference between original and rarefied samples. Increasing θ , which corresponds to increased use of abundance information, reduces the change in $BWPD_\theta$ induced by rarefaction. Phylogenetic quadratic entropy and phylogenetic entropy both show behavior similar to $BWPD_1$, with rarefaction introducing little effect.

It might be possible to formalize a statement to this effect by computing the expectation of these alpha measures under rarefaction. However, computing the expectation for $BWPD_\theta$ under rarefaction does not appear to be straightforward: the methods of *Dremin (1994)* might be applicable in this setting, however, even the integer moments of the hypergeometric distribution are complicated and the non-integer moments are bound to be very complex. We have, however, shown in the Appendix that the expectation of phylogenetic quadratic entropy under rarefaction to k sequences assigned to the tips of a

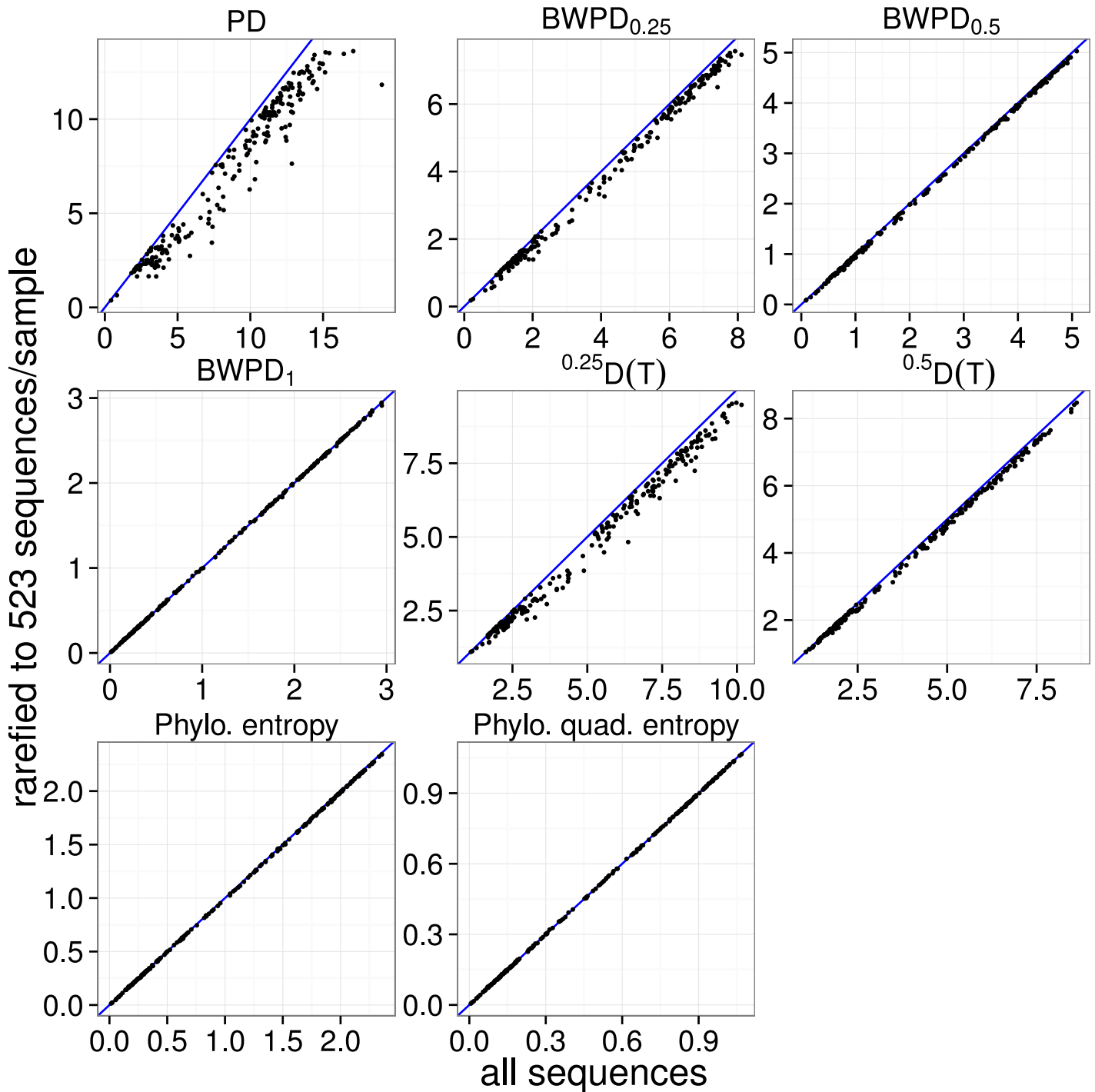


Figure 3 Comparison of rarefied and unrarefied values of various phylogenetic alpha diversity measures as applied to the vaginal dataset. The value of six alpha measures for each specimen using all available sequences is plotted on the x -axis. The value of the alpha measures for each specimen after a single rarefaction to 523 sequences (the smallest sequence count across specimens) is plotted on the y -axis. The $y = x$ line is shown in blue.

Table 3 Predictive accuracy of each measure in the oral dataset and *p*-value from an ANOVA stratified by disease status and sampling site.

Measure	Diseased status accuracy	ANOVA <i>p</i> -value	Mean rank
Phylo. entropy	0.791	4.97E−09	1.0
BWPD _{0.5}	0.782	6.50E−09	2.0
BWPD _{0.25}	0.755	7.16E−08	4.0
Phylo. quad. entropy	0.770	2.47E−07	4.0
Simpson (Family)	0.776	1.45E−06	4.0
^{0.5} D(T)	0.734	4.74E−06	6.5
^{0.25} D(T)	0.735	4.33E−05	7.0
PD _u	0.691	6.37E−06	8.5
Shannon (Family)	0.734	5.32E−05	8.5
BWPD ₁	0.698	3.57E−04	9.5
Chao1 (OTU)	0.685	9.94E−04	11.0
ACE (OTU)	0.682	1.30E−03	12.0
Simpson (OTU)	0.676	2.39E−02	13.5
Shannon (OTU)	0.672	1.31E−03	14.0
Chao1 (Family)	0.674	2.64E−01	15.0
ACE (Family)	0.663	1.82E−01	15.5

phylogenetic tree is

$$\mathbb{E}[\text{PQE}_k] = \frac{k-1}{kn(n-1)} \sum_i \ell_i d_i (n - d_i)$$

where d_i is the number of sequences falling below edge i and ℓ_i is the length of edge i . This is almost identical to the unrarefied value of phylogenetic quadratic entropy, i.e.

$$\text{PQE} = \frac{1}{n^2} \sum_i \ell_i d_i (n - d_i).$$

Thus it is not surprising to see that the expectation of PQE under rarefaction is very close to the original value (Fig. S3) for reasonably large k and n .

Oral microbiome

As previously observed by *Griffen et al. (2011a)*, we find generally higher diversity in samples from dysbiotic patients (Fig. 4). We evaluated the ability of each alpha diversity measure to predict whether a sample came from an individual with periodontitis, regardless of sample collection site, using the above methods.

In the oral dataset, phylogenetic alpha diversity measures incorporating abundance gave the best predictions of community state (Table 3, Fig. 4). In contrast, classical phylogenetic diversity performed less well. These results were almost identical in terms of rank order after applying additional quality filtering steps to correct sequencing errors and remove potentially chimeric sequences (Table S1).

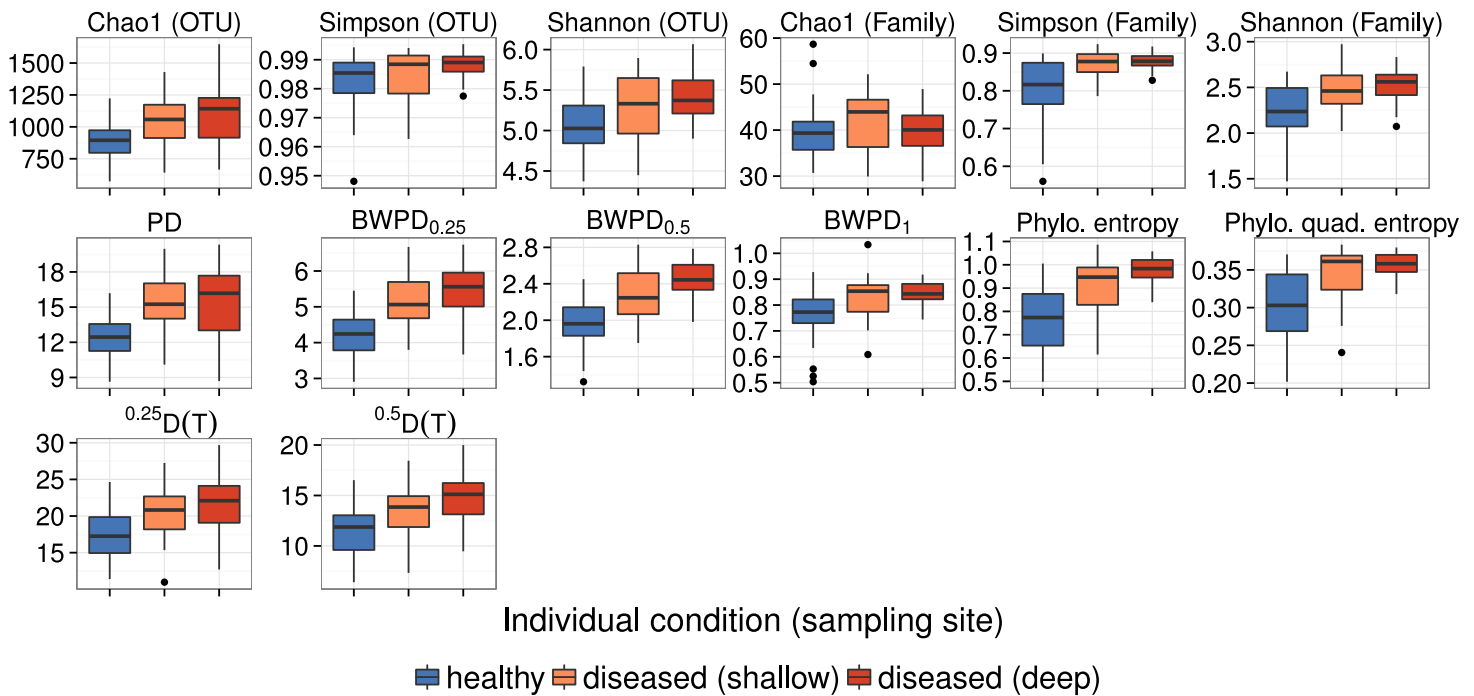


Figure 4 Comparison of diversity between samples from healthy controls, healthy sites of dysbiotic patients, and dysbiotic sites of dysbiotic patients on the oral dataset, using different measures of alpha diversity. “Shallow” means a shallow pocket between tooth and gum tissue, while “deep” means a sample from a deep pocket between gum tissue that has separated from its tooth. Top row: cluster-based methods. Bottom rows: phylogenetic methods.

OTU-based methods and phylogenetic methods are not as separated in a hierarchical clustering as for the vaginal dataset (Fig. S6). However, many of the same pairings are present in both clusterings: $BWPD_{0.5}$ with phylogenetic entropy, $BWPD_1$ with quadratic entropy, Simpson with Shannon, and ACE with Chao1. Interestingly, PD_u , $BWPD_{0.25}$, and the ${}^qD(T)$ measures all cluster with the discrete richness measures ACE and Chao1.

Like the vaginal dataset, incorporating abundance information decreases the effect of rarefaction on $BWPD_\theta$ values (Figs. S4 and S5).

Skin microbiome

To further assess resolution and robustness of phylogenetic diversity measures, we considered skin microbiome data from a study by *Oh et al. (2012)*. This study tracked the changes of the skin microbiome through “Tanner” developmental stages of adolescence (*Tanner & Whitehouse, 1976*). Because there are five Tanner stages, and they do not have a monotonic relationship with skin microbiome diversity (*Oh et al., 2012*), we focused on ANOVA p -values to see if the diversity measurements had small within-stage heterogeneity compared to between-stage heterogeneity. To compare the ANOVA p -values associated with the diversity measurements across the various datasets, we ranked the p -value of the diversity measures from lowest to highest for each dataset individually. We averaged these ranks to gain an overall measure of performance. The results again show phylogenetic

Table 4 ANOVA *p*-values for various diversity statistics applied to the skin microbiome data of Oh *et al.* (2012). Rows are ordered by increasing mean rank across sites.

	Ac	N	Pc	Vf	Mean rank
PD _u	3.34e−02	4.94e−03	3.03e−03	2.28e−04	4.50
BWPD _{0.25}	4.22e−02	1.32e−03	6.37e−03	5.86e−04	5.25
BWPD _{0.5}	8.54e−02	9.85e−05	3.65e−02	5.85e−03	6.00
Shannon (OTU)	6.61e−02	9.48e−02	9.65e−02	1.05e−05	6.50
Chao1 (OTU)	8.00e−02	6.46e−03	3.98e−03	3.18e−03	6.75
Phylo. quad. entropy	2.52e−01	1.12e−05	4.99e−01	1.67e−01	7.50
Phylo. entropy	1.37e−01	1.15e−03	1.55e−01	2.09e−02	7.75
^{0.5} D(T)	8.91e−01	5.63e−04	3.84e−03	9.09e−01	8.25
^{0.25} D(T)	7.00e−01	2.27e−03	2.35e−03	9.41e−01	8.50
BWPD ₁	3.09e−01	5.95e−05	6.65e−01	5.41e−01	8.50
⁰ D(T)	4.42e−01	1.05e−02	1.38e−03	9.38e−01	8.75
Simpson (OTU)	9.38e−02	4.01e−01	8.49e−01	1.01e−04	8.75

Notes.

The same site abbreviations are used as in their paper: Ac, antecubital fossa; N, nares; Pc, popliteal fossa; Vf, volar forearm.

measures generally performing better than OTU-based measures (Table 4). This finding holds true even after removing potential chimeras (Table S1). In this case, a light weighting or no weighting of phylogenetic diversity by abundance performed better than full abundance-weighting.

Applications summary

In all three of the datasets investigated, abundance-weighted phylogenetic diversity measures showed good performance to distinguish between community states: between “normal” and dysbiotic samples in the oral and vaginal microbiomes, and between developmental stages in the skin microbiome. Notably, the best distinguishing measure in each dataset was phylogenetic; in addition BWPD_{0.25} and BWPD_{0.5} were the only measures that were in the top four for all datasets. The result that partial abundance weighting performs well corresponds to analogous results for beta diversity, where an intermediate exponent for “generalized UniFrac” was the most powerful (Chen *et al.*, 2012).

DISCUSSION

Phylogenetic alpha diversity measures were more closely related to community state than were discrete measures based on OTU clustering for the datasets investigated here. This result is especially interesting given that the Simpson index, the Shannon index, or counting applied to OTU tables are very common ways of characterizing microbial diversity (Fierer *et al.*, 2007; Grice *et al.*, 2009; Hill *et al.*, 2003; Dethlefsen & Relman, 2011). As also noted by Aagaard *et al.* (2012), we find that measurements of diversity using taxonomic classification can be useful in describing communities, and in fact perform much better than the same measurements of diversity applied to OTU counts; however, this approach requires a taxonomically well characterized environment. Our

results can be viewed as an experimental confirmation of the notion that incorporating similarity between species is important to get sensible measures of diversity, which has been advocated by many, including most recently by [Leinster & Cobbold \(2012\)](#).

We find that classical phylogenetic diversity is sensitive to sampling depth, underestimating the true value in small samples. Biases have also been described for diversity measures using OTU tables ([Gihring, Green & Schadt, 2012](#)). In contrast, we observe that some abundance-weighted phylogenetic measures are relatively robust to varying levels of sampling.

These results did not appear to be the result of sequencing issues. In principle, OTU methods could have performed badly because of error-prone and chimeric sequences inflating the number of OTUs. Although this is a real danger for OTU quantification, in this study its impact appears to be limited—similar results were obtained with the oral data after de-noising and chimera removal and the skin data (which used Sanger sequencing) after chimera removal.

We note that on our data, non-phylogenetic measures applied to family level taxonomic groupings are generally more discriminating than the corresponding measures applied to OTUs. This may be because our sequences are from the human microbiome, and taxonomic classification is especially well-developed in that setting; in particular the taxonomic names may already be defined in a way that corresponds to dysbiosis. Thus this particular difference may not continue to be true in a taxonomically less-well-characterized environment.

As of the publication of this paper, no abundance-weighted phylogenetic alpha diversity measures are implemented in either *mothur* ([Schloss et al., 2009](#)) or *QIIME* ([Caporaso et al., 2010](#)), two of the most popular tools for analysis of microbial ecology data. Although the fact that abundance-weighted phylogenetic diversity measures performed very well for the three datasets investigated here does not imply that they are best in general, we suggest that abundance-weighted phylogenetic measures be given greater consideration for microbial ecology studies. For this to happen, implementations in commonly used microbial ecology software packages will be needed, in addition to our implementation and that of the *picante* R package ([Kembel et al., 2010](#)).

ACKNOWLEDGEMENTS

The authors would like to thank Steven Kembel for encouragement and guidance, Steven N. Evans for probability consultation, and David Nipperess for an interesting dialog concerning phylogenetic diversity and rarefaction. The Segre lab at the NIH, in particular Sean Conlan and Julia Oh, were very generous and helpful with the skin data. This work would not have been possible without an ongoing collaboration with David Fredricks, Noah Hoffman, Martin Morgan, and Sujatha Srinivasan at the Fred Hutchinson Cancer Research Center.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by NIH R01 AI038518 and startup funds from the Fred Hutchinson Cancer Research Center. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

NIH R01 AI038518.

Fred Hutchinson Cancer Research Center.

Competing Interests

The authors declare no competing interests.

Author Contributions

- Connor O. McCoy and Frederick A. Matsen IV conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper.

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.157>.

REFERENCES

- Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, Raza S, Rosenbaum S, Van den Veyver I, Milosavljevic A, Gevers D, Huttenhower C, Petrosino J, Versalovic J. 2012. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS ONE* 7(6):e36466 DOI 10.1371/journal.pone.0036466.
- Allen B, Kon M, Bar-Yam Y. 2009. A new phylogenetic diversity measure generalizing the Shannon index and its application to phyllostomid bats. *The American Naturalist* 174(2):236–243 DOI 10.1086/600101.
- Amsel R, Totten PA, Spiegel CA, Chen K, Eschenbach D, Holmes KK. 1983. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *The American Journal of Medicine* 74(1):14–22 DOI 10.1016/0002-9343(83)91112-9.
- Barker GM. 2002. Phylogenetic diversity: a quantitative framework for measurement of priority and achievement in biodiversity conservation. *Biological Journal of the Linnean Society* 76(2):165–194 DOI 10.1111/j.1095-8312.2002.tb02081.x.
- Canty A, Ripley BD. 2012. boot: Bootstrap R (S-Plus) Functions. R package version 1.3-5. Available at <http://CRAN.R-project.org/package=boot> (accessed 3 September 2013).
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows

- analysis of high-throughput community sequencing data. *Nature Methods* 7(5):335–336 DOI 10.1038/nmeth.f.303.
- Chao A. 1984.** Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11(4):265–270.
- Chao A, Lee SM. 1992.** Estimating the number of classes via sample coverage. *Journal of the American Statistical Association* 210–217 DOI 10.1080/01621459.1992.10475194.
- Chao A, Chiu CH, Jost L. 2010.** Phylogenetic diversity measures based on Hill numbers. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365(1558):3599–3609 DOI 10.1098/rstb.2010.0272.
- Chen J, Bittinger K, Charlson ES, Hoffmann C, Lewis J, Wu GD, Collman RG, Bushman FD, Li H. 2012.** Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatics* 28(16):2106–2113 DOI 10.1093/bioinformatics/bts342.
- Davison AC, Hinkley DV. 1997.** *Bootstrap methods and their applications*. Cambridge: Cambridge University Press.
- Dethlefsen L, Relman DA. 2011.** Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National Academy of Sciences of the United States of America* 108(Supplement 1):4554 DOI 10.1073/pnas.1000087107.
- Dremin IM. 1994.** Fractional moments of distributions. *JETP letters c/c of Pis'ma v zhurnal eksperimental'noi teoreticheskoi fiziki* 59:585–585.
- Edgar RC. 2010.** Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19):2460–2461 DOI 10.1093/bioinformatics/btq461.
- Faith DP. 1992.** Conservation evaluation and phylogenetic diversity. *Biological Conservation* 61(1):1–10 DOI 10.1016/0006-3207(92)91201-3.
- Fierer N, Breitbart M, Nulton J, Salamon P, Lozupone C, Jones R, Robeson M, Edwards RA, Felts B, Rayhawk S, Knight R, Rohwer F, Jackson RB. 2007.** Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Applied and Environmental Microbiology* 73(21):7059–7066 DOI 10.1128/AEM.00358-07.
- Fine PVA, Kembel SW. 2011.** Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. *Ecography* 34(4):552–565 DOI 10.1111/j.1600-0587.2010.06548.x.
- Fredricks DN, Fiedler TL, Marrasso JM. 2005.** Molecular identification of bacteria associated with bacterial vaginosis. *New England Journal of Medicine* 353(18):1899–1911 DOI 10.1056/NEJMoa043802.
- Gihring TM, Green SJ, Schadt CW. 2012.** Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. *Environmental Microbiology* 14(2):285–290 DOI 10.1111/j.1462-2920.2011.02550.x.
- Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA. 2009.** Topographical and temporal diversity of the human skin microbiome. *Science* 324(5931):1190–1192 DOI 10.1126/science.1171700.
- Griffen AL, Beall CJ, Campbell JH, Firestone ND, Kumar PS, Yang ZK, Podar M, Leys EJ. 2011a.** Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. *The ISME Journal* 6:1176–1185 DOI 10.1038/ismej.2011.191.

- Griffen AL, Beall CJ, Firestone ND, Gross EL, DiFranco JM, Hardman JH, Vriesendorp B, Faust RA, Janies DA, Leys EJ. 2011b. CORE: a phylogenetically-curated 16S rDNA database of the core oral microbiome. *PLoS ONE* 6(4):e19051 DOI 10.1371/journal.pone.0019051.
- Harrell FE Jr. 2012. Hmisc: Harrell Miscellaneous. R package version 3.9-3. Available at <http://CRAN.R-project.org/package=Hmisc> (accessed 3 September 2013).
- Hill TCJ, Walsh KA, Harris JA, Moffett BF. 2003. Using ecological diversity measures with bacterial communities. *FEMS Microbiology Ecology* 43(1):1–11 DOI 10.1111/j.1574-6941.2003.tb01040.x.
- Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26(11):1463–1464 DOI 10.1093/bioinformatics/btq166.
- Leinster T, Cobbold CA. 2012. Measuring diversity: the importance of species similarity. *Ecology* 93(3):477–489 DOI 10.1890/10-2402.1.
- Matsen FA, Gallagher A. 2012. Reconciling taxonomy and phylogenetic inference: formalism and algorithms for describing discord and inferring taxonomic roots. *Algorithms for Molecular Biology* 7(1):8 DOI 10.1186/1748-7188-7-8.
- Matsen FA, Kodner RB, Armbrust E. 2010. pplacer: linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. *BMC Bioinformatics* 11(1):538 DOI 10.1186/1471-2105-11-538.
- McDonald D, Clemente JC, Kuczynski J, Rideout JR, Stombaugh J, Wendel D, Wilke A, Huse S, Hufnagle J, Meyer F, Knight R, Caporaso JG. 2012. The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. *Journal of Nanobiotechnology Giga Science* 1(1):1–6 DOI 10.1186/2047-217X-1-7.
- Nugent RP, Krohn MA, Hillier SL. 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of Clinical Microbiology* 29(2):297–301.
- Oh J, Conlan S, Polley E, Segre JA, Kong HH. 2012. Shifts in human skin and nares microbiota of healthy children and adults. *Genome Medicine* 4(10):1–11 DOI 10.1186/gm378.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin R, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2012. vegan: Community Ecology Package. R package version 2.0-4. Available at <http://CRAN.R-project.org/package=vegan> (accessed 3 September 2013).
- Pardi F, Goldman N. 2007. Resource-aware taxon selection for maximizing phylogenetic diversity. *Systematic Biology* 56(3):431–444 DOI 10.1080/10635150701411279.
- Price MN, Dehal PS, Arkin AP. 2010. FastTree 2—approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5(3):e9490 DOI 10.1371/journal.pone.0009490.
- R Development Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.0.1. Available at <http://www.R-project.org/>.
- Rao CR. 1982. Diversity and dissimilarity coefficients: a unified approach. *Theoretical Population Biology* 21(1):24–43 DOI 10.1016/0040-5809(82)90004-1.
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75(23):7537–7541 DOI 10.1128/AEM.01541-09.

- Shannon CE. 1948.** A mathematical theory of communication. *Bell System Technical Journal* 27(1):379–423 DOI 10.1002/j.1538-7305.1948.tb01338.x.
- Simpson EH. 1949.** Measurement of diversity. *Nature* 163(4148):688 DOI 10.1038/163688a0.
- Srinivasan S, Hoffman NG, Morgan MT, Matsen FA, Fiedler TL, Hall RW, Ross FJ, McCoy CO, Bumgarner R, Marrazzo JM, Fredricks DN. 2012.** Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS ONE* 7(6):e37818 DOI 10.1371/journal.pone.0037818.
- Tanner JM, Whitehouse RH. 1976.** Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of Disease in Childhood* 51(3):170–179 DOI 10.1136/adc.51.3.170.
- Vellend M, Cornwell WK, Magnuson-Ford K, Mooers A. 2011.** Measuring phylogenetic biodiversity. In: Magurran AE, McGill BJ, eds. *Biological diversity: frontiers in measurement and assessment*. Oxford: Oxford University Press, 194–207.
- Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007.** Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73(16):5261–5267 DOI 10.1128/AEM.00062-07.
- Warwick RM, Clarke KR. 1995.** New ‘biodiversity’ measures reveal a decrease in taxonomic distinctness with increasing stress. *Marine Ecology Progress Series* 129(1):301–305 DOI 10.3354/meps129301.